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August 28, 1997

Dr. Ron Tipper
Program Manager
ONR, Code 323
800 N. Quincy Street
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Dear Dr. Tipper:

On behalf of Dr. Lauren S. Mullineaux, please find enclosed the original and two copies of the year 2 progress report for ONR Grant N00014-95-1-0763.

If there is anything else which you require, the phone number is (508) 289-2745.

Thank you.

Sincerely,

Jane E. Marsh
Senior Staff Assistant
Biology Department

xc: J. Kleindinst, Biology Department Administrator
NRL, Washington, DC
ONR, Boston
DTIC, VA (2)

enclosures

DTIC QUALITY INSPECTED 2

AUGMENTATION AWARDS FOR SCIENCE AND ENGINEERING, RESEARCH TRAINING, (AASERT)
REPORTING FORM

The Department of Defense(DOD) requires certain information to evaluate the effectiveness of the AASERT program. By accepting this Grant, which bestows the AASERT funds, the Grantee agrees to provide the information requested below to the Government's technical point of contact by each annual anniversary of the AASERT award date.

1. Grantee identification data: (R & T and Grant numbers found on Page 1 of Grant)

- a. University Name: Woods Hole Oceanographic Institution
- b. Grant Number: N00014-95-1-0763
- c. PR Number: 22ba259
- d. P.I. Name: Lauren S. Mullineaux
- e. From: 6/1/95 - 5/31/98

NOTE: Grant to which AASERT award is attached is referred as "Parent Agreement."

2. Total funding of the Parent Agreement and the number of full-time equivalent graduate students (FTEGS) supported by the Parent Agreement during the 12-month period prior to the AASERT award date.

- a. TOTAL: \$65,000 Parent Grant No. N00014-89-J-1431

PERIOD: 1 June 1995 - 31 May 1996

- b. Number FTEGS: 0

3. Total funding of the Parent Agreement and the number of FTEGS supported by the Parent Agreement during the current 12-month reporting period.

- a. TOTAL: 0 Period: 6/1/96 - 5/31/97

- b. Number FTEGS: 0

4. Total AASERT funding and the number of FTEGS and undergraduate students (UGS) supported by AASERT funds during the current 12-month period.

- a. \$90,000 Total Award \$32,499 Year Two Period: 6/1/96 - 5/31/97

- b. Number FTEGS: One

- c. Number UGS: 0

VERIFICATION STATEMENT: I hereby verify that all students supported by the AASERT award are U.S. Citizens:


Lauren S. Mullineaux

Date: Aug 27 1997

HYDRODYNAMIC FACILITATION AND CONSTRAINT OF DISPERSAL BETWEEN ISOLATED HABITATS

A progress report for an AASERT Award to Grant N00014-95-I-0763
For the period 01 June 1996 to 31 May 1997

Submitted on 21 Aug., 1997 to the Office of Naval Research by:

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Long-Term Goals:

The long-term goals of this research program are to understand the processes that control the dispersal, gene flow and diversity of benthic organisms living in patchy, isolated habitats. We address these questions through a variety of approaches, ranging from field studies of larval dispersal to laboratory studies of molecular phylogeny. The study focused originally on seamount habitats, but has been expanded to a wide range of benthic environments due to intriguing results in the phylogenetic component.

Scientific Objective:

The specific goals of this AASERT project are to resolve two particular phylogenetic questions within the Class Anthozoa (Phylum Cnidaria) using direct sequencing techniques. Traditional, morphology-based, systematic studies of these groups have resulted in substantial controversy over their phylogenetic relationships and evolution. The first question addresses relationships within the Subclass Octocorallia; traditional ordinal divisions based on morphological traits indicate that seven distinct groups exist within the Octocorallia, but critics suggest that this may be an overestimate of genetic divergence. The second question addresses the controversy of whether two or three subclasses exist within the Class Anthozoa. This project comprises the thesis research of Ewann Berntson, a student in the WHOI/MIT Joint Program.

These objectives have diverged substantially from the original aim of using molecular studies to evaluate gene flow and dispersal of benthic species inhabiting isolated seamounts. We have deviated from this original aim for two reasons: 1) we realized that the molecular results provided a unique opportunity to address important phylogenetic questions, and 2) the two segments of the genome that we were using (mitochondrial 16S rRNA and nuclear 18S rRNA) were not sufficiently variable for population-level studies.

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Approach:

The complete 18S rRNA sequence for representative species from each anthozoan family are generated through a sequential procedure of PCR, TA-cloning and sequencing on a Licor automated sequencer. Some of these specimens can be acquired fresh via submersible, SCUBA and intertidal collecting, but many others must be obtained from museum collections. These dried and ethanol-preserved specimens require special procedures for DNA extraction, amplification and sequencing. Phylogenetic relationships are evaluated using standard algorithms for genetic relatedness and distance analyses (e.g., Kimura 2-parameter, Jin/Nei, maximum likelihood, and parsimony). The studies are being done in close collaboration with Dr. Ted Bayer (cnidarian expert at the Smithsonian Institution) and Dr. Scott France (UNH).

Tasks Completed:

To date, representative species have been obtained from 49 anthozoan families, out of a total of 64 that are important for this study. Many of these specimens were collected during two sets of Pisces V submersible cruises to Hawaiian seamounts through the NOAA Hawaiian Underwater Research Laboratory. Others were collected in shallow water or obtained from museum collections. We are continuing to search for specimens from the remaining 15 families. Full sequences of the 18S rRNA gene have been generated for 51 specimens. A procedure involving the development of octocoral-specific primers and cloning of three separate fragments of the 18S rRNA gene has been devised to sequence the problematic museum specimens.

Scientific Results:

Preliminary analyses of these anthozoan sequences do not support the traditional taxonomic classification of the octocorals at the ordinal and subordinal levels (question 1). In a few cases where confamilial species have been sequenced, some of the families appear to be monophyletic but others do not. The Order Pennatulacea, which was previously considered a well-supported group morphologically, is also proving to be polyphyletic. These observations are critical for evaluating the evolutionary relevance of family-level and higher groupings within the Octocorallia.

Preliminary results from the subclass-level analyses (question 2) show that the two orders within the Subclass Ceriantipatharia are not monophyletic. The antipatharians cluster with the hexacorals, as was suggested by the traditional two-subclass taxonomic system, but the cerianthids branch most ancestrally to all of the remaining anthozoans. The addition of more sequences from antipatharian and cerianthid species will further elucidate these relationships. This preliminary observation that the Subclass Ceriantipatharia is not a monophyletic grouping of species is perhaps the most striking result obtained in this study to date.

Significance:

These new observations on genetic relationships among species within the Class Anthozoa will bring about a substantial revision of the taxonomy of this group at the sub-class and ordinal levels. In doing so, this project will change our understanding of the evolution of this group, whose members include major components of the faunas in many patchy, isolated habitats, including seamounts and coral reefs. These observations also have direct relevance for a wide spectrum of other studies:

- 1) Dispersal - The DNA sequences generated in this project provide us with species-specific molecular "tags" which can be developed into larval probes or other methods for identifying morphologically-indistinct anthozoan larvae collected in the field.
- 2) Biodiversity and gene flow - The extraction and sequencing technique developed for preserved museum specimens opens up an enormous wealth of cnidarian specimens that can now be used for studies of biogeography and gene flow.
- 2) Environmental impact - Understanding the diversity and evolution of the Anthozoa will assist us in predicting the susceptibility of important marine communities (e.g., seamount faunas, coral reefs) to anthropogenic impact, and evaluating their resiliency in previously impacted areas.

Presentations:

Berntson, E., "Phylogenetic Relationships Within the Anthozoa (Phylum Cnidaria) As Inferred From 18S Ribosomal DNA", presented at the Evolution Meetings held in Boulder, CO (June, 1997)

Publications in preparation (tentative titles and authors):

Berntson, E.A. and S. C. France. Generating DNA sequence information from museum collections of cnidarian specimens. For *Molecular Marine Biology and Biotechnology*.

Berntson, E. A., S. C. France and F. M. Bayer, Phylogenetic relationships within the Anthozoa (Phylum Cnidaria) as inferred from 18S ribosomal DNA sequences. For *Evolution or Molecular Marine Biology and Biotechnology*.

Berntson, E. A., S. C. France and F. M. Bayer, Genetic variation within the Subclass Octocorallia (Cnidaria: Anthozoa) based on 18S ribosomal DNA. For *Evolution or Molecular Marine Biology and Biotechnology*.

France, S. C., and E. A. Berntson. Diversity and distribution of octocorals and other species on seamounts near Hawaii." For *Deep-Sea Research*.

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13. ABSTRACT (Maximum 200 words) The specific goal of this AASERT project is to use molecular techniques to resolve phylogenetic questions within the anthozoans (phylum Cnidaria). The study focuses on current controversies over the ordinal divisions within the Subclass Octocorallia, and the subclass divisions within the Class Anthozoa. To date, full sequences of the 18S rRNA gene have been generated for 51 specimens collected fresh or obtained preserved from museum collections. Preliminary analyses of octocoral sequences do not support the traditional taxonomic classification of this group at the ordinal and subordinal levels. The order Pennatulacea, which was previously considered a well-supported group morphologically, is proving to be polyphyletic, as are some of the families. Results from the subclass-level analyses show that the two orders within the subclass Ceriantipatharia are not monophyletic. These new observations on genetic relationships among species within the class Anthozoa will bring about a substantial revision of the taxonomy of this group at the sub-class and ordinal levels. In doing so, this project will change our understanding of the evolution of this group, whose members include major components of the faunas in many patchy, isolated habitats, including seamounts and coral reefs.				
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